A NEW ABSOLUTE-SCALE SMALL-ANGLE X-RAY SCATTERING INSTRUMENT

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ABSTRACT

A small-angle X-ray scattering apparatus is described which is based on a Guinier focusing arrangement, utilizing a fine-focus tube, a Johann-type curved-crystal monochromator, two goniometer-mounted beam-defining slits and proportional detection. It differs from previously described instruments in important respects. Using a horizontal goniometer, it affords ease of access and mechanical stability to mounted parts, such as slits and beam stop. The major instrument assemblies--the horizontal tube housing producing the vertical line-shaped beam, and the goniometer table--are mounted on a granite surface plate for stability. For ease, precision and reproducibility in alignment, fine adjustments, with micrometer heads or dial indicators where advisable, are provided for all pertinent rotational and translational motions of the various subassemblies. In particular, provision is made for fine adjustment of the goniometer as a whole with respect to the X-ray source, to facilitate threading the monochromator-focused beam through the slit system and the center of rotation of the detector arm. Maximum distance between the beam-defining slits compatible with monochromator geometry yields increased resolution. Use of a pulse-height analyzer eliminates harmonic radiation from the detection system and thus allows operation of the X-ray tube at greater intensity. Absolute measurements are made by means of calibrated beam-attenuators to refer scattered and direct-beam intensities to the same scale. An accessory is a motor-driven horizontal slit for scanning the vertical beam profile to validate assumptions used in analysis of the data, i.e., slit-desmearing to refer the slit-produced scattering to a theoretical point source. Automatic step scanning with

punched-tape output is provided for automatic data processing. The facility for symmetrical scanning provides a check on proper alignment as well as on source stability and system geometry during the extended scans required for weakly scattering systems, such as dilute solutions of biological macromolecules. With the use of this instrument, radii of gyration were determined for several globular proteins (β-lactoglobulin, ribonuclease, lysozyme) and values in agreement with those in the literature were obtained.

INTRODUCTION

Small-angle X-ray scattering has been employed for many purposes and in the study of many types of system, e.g., for particle and pore sizes in catalysts, particle sizes and clustering in alloys, glasses and ceramics, observation of critical phenomena, colloidal micelles in solution, synthetic polymers and biopolymers. The variety of parameters obtainable by the method (radius of gyration, particle size, pore size, molecular weight, mass per unit length in linearly ordered systems, surface-to-volume ratio or specific surface, particle volume, internal hydration, and others) has made it sufficiently attractive to lead to a considerable number of instruments constructed for it. These instruments, as well as much of the theory, have been reviewed by Guinier and Fournet,[1] Beeman et al.[2] and others, and very complete bibliographies of the literature have been given by Yudowitch in Guinier and Fournet[1] and by Goldmann.[3] More recent instruments have been described by Luzzati at al.,[4] Brumberger and Deslattes,[5] Renouprez et al.,[6] Bonse and Hart,[7] Koffman,[8] and Kavesh and Schultz.[9]

Experimental Obstacles

In the study of proteins in dilute solution in particular, one is faced with difficult experimental requirements. Since scattered intensities generally are weak, counting statistics require long counting times, in the case of step scanning, or very slow scans, in the case of continuous scanning. Either way, the time for a complete scan may range from 8 to 24 hours, during which the primary-beam intensity is expected to remain constant. This is generally attempted by the use of very stable sources, including highly stabilized power supplies, air conditioning and cooling-water temperature control; even so, primary-beam intensity fluctuations are not always eliminated (cf. Baker et al.,[10] who correlated intensity variations with barometric pressure changes). To the extent that high-intensity primary beams (by virtue either of high-intensity sources or of collimating systems passing a relatively wide beam) will give increased scattered intensities, the time requirements will be ameliorated.

Another approach would be to utilize a record of monitored primary-beam intensities to apply point-by-point normalization to the measured scattered intensities. There are practical difficulties in this approach which appear to have prevented it from being applied routinely so far.

Under these circumstances it would seem desirable to have some other check on the stability during a scan. Those instruments which allow scanning on either side of the zero angle, assuming they are in perfectly symmetrical alignment, afford this possibility and provide an immediate duplicate determination as well, although of course at the cost of approximately doubling the time required. A check for symmetry at the end of each complete scan then requires no more than a translucent chart record paper and an illuminated viewing box.

Other experimental difficulties relate to the need for measurements at the very small angles corresponding to large repeating units or large molecular weights. Although collimating systems yielding high primary-beam intensities will result in better counting statistics and shorter scanning times, a broader primary beam generally will have tails which may be high compared to the scattered intensities at somewhat wider angles, particularly when it is remembered that scattered intensities are lower than direct-beam intensities by a factor, typically, of 10^6. Provisions for precise and reproducible alignment of all the collimating system components and for elimination of parasitic scattering due to various causes are indispensable. Otherwise it is not feasible to find a suitable compromise, for a given sample, between the conflicting demands of high intensity and high resolution.

Further requirements result from the need to measure primary-beam intensities on the same scale as scattered intensities, if the instrument is to give the absolute-scale measurements required for determination of molecular weights and other parameters. The problem has been summarized by Kratky et al.[11] Methods used for this purpose fall into two broad categories: 1. Use of standard scatterers whose scattering either is calculated from basic data (Beeman et al.,[12] Weinberg[13]) or is determined by calibration with reference to one of the other methods (Kratky[11]); 2. Use of attenuators, either by fractional-time sampling of the primary beam (rotating disk with hole, Kratky[14]) or by filter-type foil attenuation (Luzzati,[15] Weinberg,[13] Damaschun and Müller[16]).

Problems with Existing Instruments

Instruments described in the literature sometimes achieve very high performance with respect to one or more of the desirable requirements by accepting severe restrictions with respect to others.

Some of the most attractive-appearing designs may be described in detail, but beyond that first description in the literature one often finds little further publication of data obtained by their use.

Instruments frequently fall short of the ideal in one or more of the following respects.

1. The advantages of a symmetrical primary beam and a symmetrical scan are relinquished, in return for the possibility of eliminating parasitic scattering from the beam-defining slit edges to a very high degree; more precisely, the slit-produced penumbra of the source focal spot is eliminated almost entirely on one side of the zero angle, in return for covering up the beam on the other side of zero angle.

2. Instruments use a goniometer on a horizontal axis, with the arm moving in a vertical plane. This may result in greater compactness and thus possibly greater rigidity, as well as less space

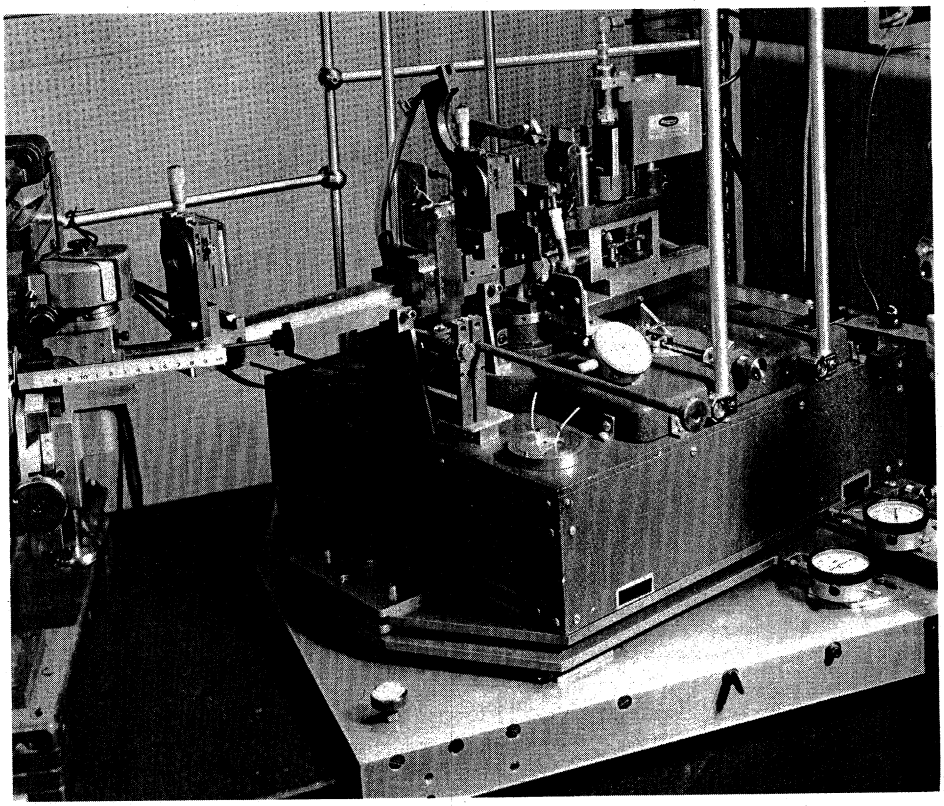

Figure 1. Small-angle X-ray scattering instrument.

required, but it may also result in lessened accessibility and may make mounting of various accessories more difficult and more tenuous. A vertical-axis goniometer, with the arm moving in a horizontal plane, lends itself to a more open construction, with ample room for access and for the attachment of components to the stationary horizontal goniometer table.

3. Instruments using pure slit collimation have to discard most of the divergent beam issuing from the source in order to obtain a well-defined beam. To increase intensity, high-intensity rotating-anode X-ray tubes are sometimes employed.

4. Instruments using a curved crystal for primary-beam monochromatization change the divergent beam coming from the source into a converging beam passing through the sample and do not need to suffer a great loss of intensity from collimating slits, but the reflection at the crystal face itself is accompanied by substantial losses.

5. Most instruments are not adapted to make absolute measurements routinely, if at all. The use of gaseous standard scatterers is not a simple procedure; solid or liquid secondary standards can be used routinely but depend on standardization in an instrument of identical geometry; the rotating-disk method of Kratky is too complex to be used routinely; foil attenuators require a set of carefully calibrated high-grade foils, and provisions to accommodate them.

6. Some instruments are suitable primarily for very small angles, and their range is correspondingly small (± 3° or less), whereas an all-purpose instrument may be required to take measurements to ± 8°.

APPARATUS

On the basis of the above considerations, the instrument shown in figures 1 and 2 was designed, constructed, aligned and tested.

Construction

The two major assemblies of the instrument, the horizontal tube housing with attached monochromator, and the goniometer with slits and detector, are mounted on a 24" x 48" granite surface plate (SP) supported by a steel frame. Some inconvenience in securing components to this massive slab was felt to be a small price to pay for obtaining the utmost in rigidity and insuring maintenance of alignment between these two assemblies.

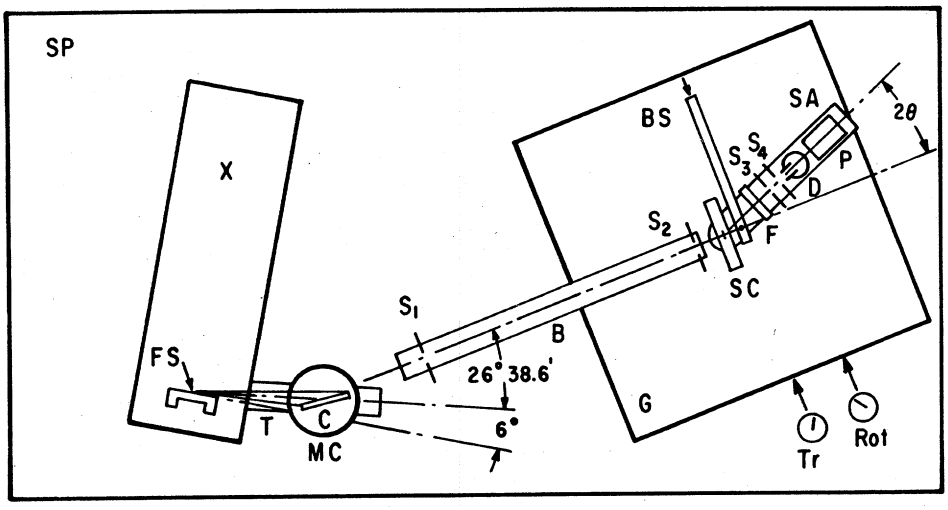

Figure 2. Diagram of scattering instrument.

The X-ray source (X) is a fine-focus copper-target Philips (Eindhoven)* four-window diffraction tube of 1200 watt capacity (PW 2113), mounted in a horizontal tube housing in such a way that a vertical beam issues from one of the line-focus windows and, viewed at a 6° take-off angle, the focal spot (FS) has effective dimensions of 0.04 mm x 8 mm. The ordinarily cantilevered end of the tube housing is further supported by a rigid welded-steel column attached to the foot of the L-shaped housing brace.

Attached to this column also is the monochromator support assembly, which includes a set of four screw motion tracks: circular, vertical and horizontal tracks in planes transverse to the 6° take-off direction, and a longitudinal track (T) at the 6° angle, whose axis is centered on the tube focal spot. This track carries the monochromator housing (MC), which has a rotatable support table with a lockable coarse adjustment (Compagnie Générale de Radiologie). Once this adjustment is locked, a fine motion by means of a differential tangent screw allows adjustment to within 0.1 minute of arc. This table was originally designed for a magnetic crystal-bending clamp of the same manufacturer; since a non-magnetic demountable

* Mention of specific firms or products does not imply endorsement by the U. S. Department of Agriculture to the possible exclusion of others not mentioned.

clamp of another manufacturer (Etablissements Beaudouin) was found more practical for permitting a choice of special crystals, the table has been equipped with a suitable hold-down clamp.

The focusing geometry (essentially that of Guinier-Luzzati[1,4]) is based on a flat-cut circularly bent quartz crystal in the Johann[17] configuration, utilizing reflection from the 1011 planes.[18,19] The crystal faces are cut at an 8° angle to the reflecting planes; the crystal (C) is elastically bent to a radius of 1300 mm. This asymmetry results in a favorable geometry in which the monochromator is very close to the source, thus subtending a relatively large angle and collecting an intense divergent beam; at the same time it is relatively far from the detector, thus allowing room for a large separation between the two beam-defining slits, with a consequent high degree of elimination of parasitic scattering from crystal, crystal clamp and the edges of the first slit itself. The quartz plates, 13 mm x 40 mm in aspect and 0.3 mm thick, supplied by Dr. Steeg and Reuter G.m.b.H., must be individually selected, since their quality is crucial, and must be carefully mounted in the bending clamp by trial and error. The clamp, in turn, must be carefully mounted so that the center of the crystal face passes through the center of rotation of the monochromator table. Properly adjusted, the monochromator will isolate the α_1 line from the Cu-Kα doublet at a Bragg angle of 13° 19.3'. The criteria for crystal quality, correct mounting and proper adjustment are the intensity and homogeneity of the monochromatic beam as viewed on a fluorescent screen, and the shape of its scanned profile as recorded on a chart.

The two beam-defining slits (S_1 and S_2) are mounted on an optical bench (B) supported on the goniometer table (G). This bench is rotatable about the center of rotation of the scanning arm (which is also the center of the sample cell), and the median line of each of these slits is adjustable to lie in the vertical plane passing through this center of rotation.

The two slit assemblies, especially designed and supplied by W. W. Beeman, are compact devices embodying transverse horizontal and rotational adjustments. The 35-mm long tantalum slit jaws open and close symmetrically by means of a micrometer adjustment. The slit assemblies are mounted on carriages slidable on the bench. The fine adjustment and the quality of the slit jaws, particularly of the second slit, are extremely critical to the apparatus.

The two scanning slits (S_3 and S_4) mounted on the scanning arm (SA), define the scattering angle, 2θ, at which intensities are observed. The first of these (S_3) is the receiving slit and determines resolution as well as intensity seen by the detector (D), also mounted on the arm. The second slit (S_4), an anti-scatter

slit, limits the radiation seen by the detector to the direction coming from the sample. Without it, parasitic scattering originating from various components and from irradiated air in the vicinity of this direction would pass through the receiving slit and reach the detector. Because of space limitations the original, very compact Philips slits are used. These are exchangeable fixed-width slits with molybdenum jaws. Adjustments originally were limited to transverse quasi-translations of each (actually small rotations about a vertical axis), as well as a coarse longitudinal translation of the two slits and the detector as a unit along the scanning arm. This latter motion has been modified to a fine adjustment. A trimming adjustment has been added which allows these same three components (scanning slits and detector) to be rotated as an entity with respect to the scanning arm.

A transverse fifth slit (shown in figure 1, but not in figure 2) is a motor-driven vertical-scanning slit, used for measuring the vertical beam profile. This information is useful in determining the proper weighting function to be used in desmearing the

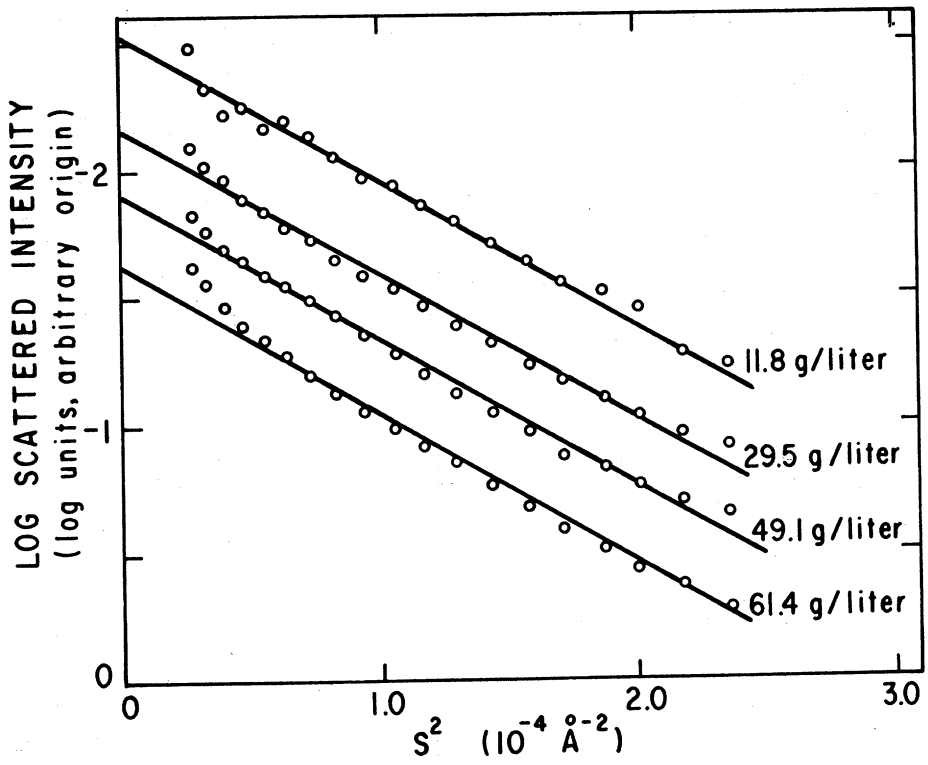

Figure 3. Guinier plots for β-lactoglobulin (pH 5.2 sodium acetate buffer, $\Gamma/2 = 0.1$).

measured scattered intensities, i.e., in deconvoluting observed intensities derived from a line source to theoretical intensities ascribable to the point source on which scattering theory is based. (If the assumption of infinite slit height holds (Guinier,[1] Luzzati,[15] Kratky et al.[20]) this correction is particularly simple.) This measurement, furthermore, supplies a check on parallelism of all four longitudinal slits with one another and with the monochromatized beam. The preferred location for this slit would have been between sample and receiving slit; because of space limitations, however, it is removably mounted on the optical bench.

The sample cell (SC) is held by a removable sample holder having translational adjustments to permit centering on the axis of rotation. A θ-motion or a fixed position may be selected. The liquid-sample cell, with window height of 30 mm and volume of 1/6 ml, consists of a 1 mm Teflon spacer (approximately the optimum sample thickness) clamped between two approximately 0.0007" thick mica windows by means of the cell frame, together with access passages for introducing and removing samples. The only materials in prolonged contact with sample solutions are Teflon and mica. Window thickness represents a compromise between minimizing X-ray attenuation and window fragility.

A beam stop (BS), consisting of one of a set of gauged wires, is held by clamps in the C-frame of the beam-stop holder, which in turn is supported by a dove-tail slide attached to the goniometer table. Sensitive transverse motion of the stop is effected by a two-speed screw (coarse and fine motion combined in one screw) bearing against the slide. The motion is indicated by a dial indicator for the purpose of reproducibility.

Filters are built up of high-quality nickel foils of the required thickness, mounted in frames held removably in a filter holder attached to the scanning arm in front of the receiving slit. A set of filters of varying thicknesses (filter factors between 1.2 and 800) was constructed and calibrated.

The entire goniometer box, supporting the components described above as well as the motor, gears and controls inside the box, is mounted on a set of two 5/16"-thick ground steel plates and a 1/2"-thick aluminum jig plate. The aluminum plate is clamped to the surface-plate table. The lower of the two steel plates is supported on the aluminum plate by a dove-tail slide and by steel balls in the form of ball-bearing parallels, allowing a sliding motion transverse to the optical bench. The upper of the two steel plates is supported on the lower by a pivot pin adjustable to be located perpendicularly underneath slit 1, as well as by steel balls like the other plate, allowing rotation about the median line of this slit as an axis. The translational adjustment is controlled

by a differential screw, the rotational adjustment by another two-speed screw, and both motions are indicated by dial indicators (Tr, Rot) to facilitate aligning the goniometer. By these means the vertical plane passing through the center lines of the slits and the sample may be adjusted to make the correct angle (26° 28.6', twice the Bragg angle) with the monochromator track and may thus be centered on the monochromatized beam.

The detector (D) is a sealed-window Amperex proportional counter tube with a Hamner NB-19 preamplifier, mounted on the scanning arm. Electronics consist of a Wanlass Parax power conditioner, a Fluke 412B high-voltage power supply, a Hamner NA-11 linear amplifier and a Hamner NC-11 pulse-height analyzer feeding into the ratemeter and display portions of a Philips 12206 electronic circuit panel, and from there into a Honeywell Electronik strip chart recorder. The goniometer may be used in the continuous mode or, under the control of the circuit panel, in a step scanning mode. With step scanning, data may be fed to a Victor Digit-Matic tape printer and, through an interface, into a Friden SP-2 tape punch.

Alignment

The monochromator is aligned with the aid of a fluorescent screen. The criterion of longitudinal alignment (cf. Guinier[21]) is the sharp disappearance of the beam without shifting, upon a slight rotation of the crystal in either direction away from the position of maximum intensity and homogeneity. At any but the correct distance between tube and monochromator, rotating the crystal

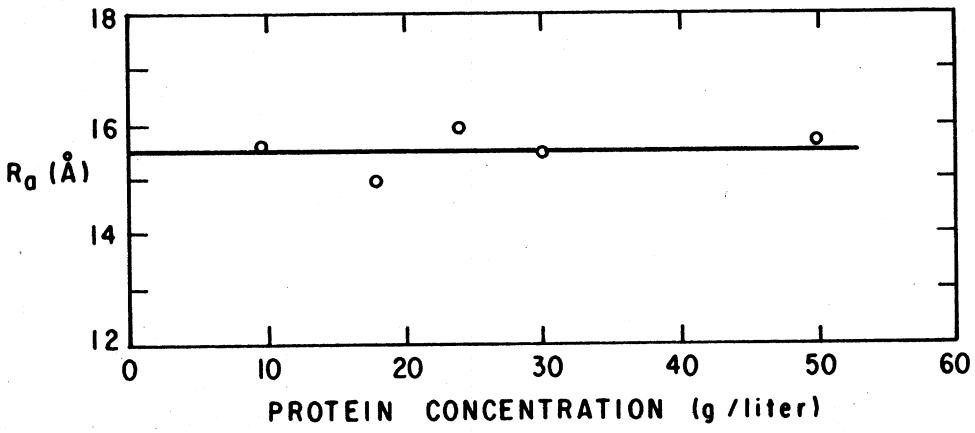

Figure 4. Radius of gyration vs. concentration, for lysozyme (pH 6.6 sodium phosphate buffer, $\Gamma/2 = 0.1$).

either way produces some vertical motion of the fluorescent spot before it disappears.

The goniometer is lined up with the monochromatized beam by the use of an auxiliary slit in the sample position. This slit is first precisely adjusted to coincide with the scanning center of rotation. With slit 1 on the bench but slit 2 removed, successive translational and rotational adjustments of the goniometer platforms guide the beam first through slit 1 and then through the scanning center. With the scanning slits removed, the goniometer can now be zeroed.

The receiving and anti-scatter slits are next replaced and aligned in turn, and the auxiliary slit is then removed. Slit 2 is replaced last and is centered and opened so as to barely graze the direct beam. Exceedingly fine adjustments of this slit are required to produce a symmetrical direct beam.

RESULTS

Radii of gyration were determined for the globular proteins β-lactoglobulin B, lysozyme and ribonuclease, each at a series of concentrations. The β-lactoglobulin B was prepared according to the method of Aschaffenburg and Drewry[22] from the milk of homozygous B/B cows. Egg-white lysozyme, 3x crystallized, was obtained from Pentex Incorporated. The ribonuclease was the salt-free bovine pancreatic product, 5x crystallized, supplied by Mann Research Laboratories, Inc.

The apparent radius of gyration, R_a, at any concentration can be obtained from the corresponding Guinier plot[1], log $j_n(s)$ vs. s^2, where $j_n(s)$ is the net normalized scattered intensity at the angle measured by $s = (2 \sin \theta)/\lambda$, θ is the Bragg angle, or one-half the scattering angle, and $\lambda = 1.5405$ Å is the wavelength of the incident copper K_{α_1} radiation. In the region of small angles, this plot approaches linearity, and the limiting slope, $(\text{slope})_o$, is related to the apparent radius of gyration, R_a, by the expression $(\text{slope})_o = -(4/3)\pi^2 R_a^2$. Extrapolation to zero concentration of a plot of R_a as a function of concentration yields the actual radius of gyration, R_o.

Guinier plots for β-lactoglobulin B (in pH 5.2 sodium acetate buffer, $\Gamma/2 = 0.1$) at several concentrations are shown in figure 3. Extrapolation to zero concentration of values of R_a derived from these plots gives $R_o = 21.0$ Å. Witz et al.[23] have reported a value of 21.7 Å.

Figure 4 is a R_a vs. concentration plot for lysozyme (in pH 6.6 phosphate, $\Gamma/2 = 0.1$), where the R_a values were calculated from slopes of Guinier plots similar to those of figure 3. Extrapolation yields $R_0 = 15.5$ Å, compared with 15.2 Å reported by Luzzati et al.[24]

A similar study for ribonuclease (in pH 5.2 acetate buffer, $\Gamma/2 = 0.1$) gave a value of $R_0 = 14.6$ Å. From the crystallographic data of Kartha et al.;[25] approximating the shape of the ribonuclease molecule by a right parallelepiped with dimensions of 38 x 28 x 22 Å and using the expression $\sqrt{(a^2 + b^2 + c^2)/12}$ for the radius of gyration, one arrives at $R_0 = 15.03$ Å.

It is seen, therefore, that results obtained with this instrument for three different proteins are in good agreement with comparable values from the literature.

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